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Research Article

PROTECTIVE ACTIVITY OF ALPHA-PINENE AND TRANS-ANETHOLE AGAINST 3-NITRO PROPIONIC ACID INDUCED NEUROTOXICITY IN ANIMAL MODEL OF HUNTINGTON'S DISEASE

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ABSTRACT

Huntington's disease (HD) is a highly destructive autosomal dominant neurodegenerative disorder. 3-Nitropropionic acid (3-NP), model is a well-known model to induce Huntington's disease (HD) in rodents. It is a neurotoxin which causes severe neurotoxicity in the form of oxidative stress. Alpha-pinene (AP) and Trans-anethole (TA) are the natural compounds having mainly antioxidant and neuroprotective effects. In the present study, the neuroprotective effect of Alpha-pinene and on 3-NP induced oxidative stress in rat striatum was determined by behavioural and biochemical parameters. Rats were induced with 3-NP (10 mg/kg) intraperitoneally for 14 days and rats induced with 3-NP were treated with Alpha-pinene and Trans-anethole - (50 mg/kg, each, per. oral) and the combination group (AP + TA) - (25 mg/kg, each, p. o) for 14 days. Alpha-pinene and Trans-anethole have given better results in the case of body weight reduction and behavioural analysis which includes (elevated plus maze (Anxiety), forced swim test, rota rod activity (grip strength). Biochemical estimation includes (lipid peroxidation (MDA), SOD, GSH, Catalase levels), It decreases the increased level of MDA and increased the decrease level of SOD, GSH and Catalase levels), while combination effect of Alpha-pinene and Trans-anethole were found to be more effective. The present study shows that the antioxidant activity of alpha-pinene and trans-anethole may be responsible for its neuroprotective activity against 3-nitropropionic acid induced neurotoxicity in rats.

Keywords: Alpha-pinene, Trans-anethole, 3-nitropropionic acid, Neurotoxicity, oxidative stress, Huntington's disease

INTRODUCTION

Huntington's disease (HD) is an autosomal dominant highly destructive neurodegenerative disorder, characterized by motor dysfunction, emotional disturbances, abnormal involuntary movements, dementia and weight loss[1,2]. Huntington's disease is prevalent in western population, but recent reports suggest that Huntington's disease may also be prevalent in India[3,4]. Huntingtin is a protein responsible for neuronal development in embryonic stage. Huntington's disease is caused due to expansion of CAG repeats in Huntingtin (Htt) gene. The expansion of poly glutamine repeats brings about a conformational change in mutant huntingtin causing it to aggregate and form inclusions body in the inner mitochondrial membrane, results it in the dysfunction of mitochondria and oxidative stress which leads to degeneration of GABAergic striatal neurons[5].

3- Nitropropionic acid, a fungal toxin produced by *Aspergillus flavus*, has been reported to be used for producing Huntington's disease like symptoms by inhibition of mitochondrial enzyme succinate dehydrogenase. It also alters the levels of biochemical parameters and behavioural activities. Many genetic or chemically induced models have been developed in rodents to study the disease. 3-Nitropropionic (3-NP) acid is a well-known neurotoxin to induce Huntington's disease (HD) in rodents. It replicates the pathology of Huntington's disease by causing oxidative stress. Oxidative stress causes alterations in the levels of enzymatic antioxidants[6].

Alpha-pinene (Major volatile oil constituent of *Murraya koenigii*) and Trans-anethole, (Major volatile oil constituent of *Foeniculum vulgare*), which are natural compounds having, antioxidant and neuroprotective effects. It has been used as drugs against neurotoxicity produced in the form of oxidative stress which induced by 3-Nitro propionic acid. Alpha-pinene has antioxidant, GABAergic transmission potentiation effect and acetylcholinesterase inhibitor effects. While, Trans-Anethole has antioxidant, calcium channel blocker and bioavailability enhancer effects and both have sedative, anxiolytic, antidepressant and catecholamines depletory effects[7-9].

In this present study attempt has been made to evaluate the neuroprotective effect of alpha-pinene and Trans-anethole against 3-nitropropionic acid induced Huntington's disease like symptoms.

MATERIAL AND METHODS

Chemicals

3-nitropropionic acid, Alpha-pinene and Trans-anethole were purchased from Sigma- Aldrich Co. (St. Louis, USA). All other chemicals and reagents used in this study were of analytical grade.

Animals

Male Wistar rats (200-240 gm) were obtained from Animal House, of R.V. Northland Institute, Dadri, Gautam Budh Nagar, U.P. 203207. All animals were maintained in 12 h light/ dark cycle. The rats had free access to food and water. All the experiments were conducted according to the ethical rules approved by Institutional Animal Ethical Committee having

CPCSEA registration no- 1149/PO/Ere/S/07/CPCSEA, date of registration- 13/Feb/2014.

Experimental Procedure

The rats were randomly divided into five groups (6 rats each group) as below:

- Group I - Control- Rats simultaneously treated with saline – 1 ml/kg- p. o (per. oral) and saline - 1 ml/kg- i. p for 14 days.
- Group II - Rats administered with 3-Nitropropionic acid (10 mg/kg b. w.) intraperitoneally (i. p.) for 14 days.
- Group III - Rats simultaneously treated with 3-NP (10 mg/kg b. w. i. p) and Alpha-pinene (50 mg/kg, per. oral) for 14 days.
- Group IV - Rats simultaneously treated with 3-NP (10 mg/kg b. w. i. p.) and Trans-Anethole (50 mg/kg, per. oral) for 14 days.
- Group V – (Combination group) Rats simultaneously treated with 3-NP (10 mg/kg b. w. i. p.) + Alpha-pinene (25 mg/kg, per. oral) + Trans-Anethole (25 mg/kg, per. oral) for 14 days.

Measurement of Body Weight

Body weight

Body weight of the animals was recorded on day 1, 7 and day 14. Change in body weight was examined.

Behavioural analysis

Grip strength test

Grip strength was measured by suspending rat with the forepaws to a thick steel wire of 2 mm in circumference and 80 cm in length, placed 50 cm above a cushioned support. The length of the time for which rat remain suspended on wire was measured[10].

Forced swim test

Forced swim test was carried out according to the method of Porsolt *et al*[11]. The rats were placed in Plexiglas cylinders of height 20 cm and diameter 10 cm containing 15 cm water, maintained at 23-25^{0}C. Animals were released in water and the time of immobility was calculated. Animals were considered to be immobile when they swam.

Elevated plus maze test for spatial memory (Anxiety)

Briefly, the elevated plus maze consists of two opposite open arms (50 x 10 cm), crossed with two closed arms of the same dimensions with 40 cm high walls. The arms are connected with a central square (10 x 10). The animal were placed individually at one end of an open arm facing away from the central square and the time taken by the animal to move from the open arm and enter one of the closed arms will be recorded as initial transfer latency (ITL). The rat were allowed to explore the maze for 30 s after recording the initial acquisition latency and returned to its home cage[12].

Biochemical Estimation

Sample preparation for Biochemical estimation

On day 15, after behavioural assessments, animals were sacrificed by CO_2 asphyxiation method. The brains were removed, put on ice and striatum was separated. A 10% (w/v) tissue homogenate was prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 10,000 g for 15 min and aliquots of supernatant were separated and used for biochemical estimation.

Estimation of Lipid peroxidation (MDA levels)

Lipid peroxidation was measured by the method of Ohkawa *et al*. (1979)[13]. Malondialdehyde, which is a marker of lipid peroxidation, was measured at 532 nm (wave length via U.V or colorimeter or other). The values were calculated using the molar extinction coefficient. The results were expressed as nmole of MDA /g of protein.

GSH (Glutathione)

Estimation of glutathione (GSH) in striatum was done according to the method of Moron *et al*. Briefly, 5, 5'-dithiobis (2-nitrobenzoic acid) (DTNB) is reduced by –SH groups and 1 mole of 2-nitro-5-mercaptobenzoic acid is produced by per mole of SH. The intensity of released nitro mercaptobenzoic acid anion is measured at 412 nm. The results were expressed as nmoles/mg of protein[14].

Superoxide Dismutase (SOD)

SOD activity was assayed according to the method of Kakkar *et al*. (1994)[15]. The absorbance of assay mixture was recorded at 560 nm (wave length via U.V or colorimeter or other) and the results were expressed as units / mg of proteins.

Catalase

Catalase activity was assayed by the method of AebiH *et al*. (1974)[16], in which the breakdown of hydrogen peroxide (H_2O_2) was measured at 240 nm (wave length via U.V or colorimeter or other). The results were expressed as µM of H_2O_2 decomposed per milligram of protein/min.

RESULT

Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in body weight

Variation in the body weight between all the groups was evaluated by taking their weights on day 1 day 7 and day 14. Intra-peritoneal administration of 3-NP for 14 days showed significant ($p < 0.001$) decrease in body weight in Huntington's disease rats when compared with control rats. Treatment with alpha-pinene and Trans-anethole in 3-NP induced rats significantly ($p < 0.01$) improved the body weight and more significantly ($p < 0.001$) results were observed in the group receiving combination of alpha-pinene and Trans-anethole.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Behavioural analysis

Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in Grip strength

Motor coordination of all the animals was evaluated by their rota rod performances. Administration of 3-NP for consecutive 14 days significantly ($p < 0.001$) decreased motor coordination and body balance when compared to normal control rats. Treatment with alpha-pinene and Trans-anethole in 3-NP induced rats

significantly ($p < 0.01$) improved the motor coordination and more significant ($p < 0.001$) results were observed in the group receiving combination of alpha-pinene and Trans-anethole.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in memory retention (anxiety)

Effect of treatment drugs on memory retention was evaluated through the activity of animals on elevated plus maze. The time taken by the animal to move from the open arm and enter in to one of the closed arms on day 1 was recorded as initial transfer latency (ITL). The time taken by the animal to move from the open arm and enter in to one of the closed arms on day 14 was recorded as retention transfer latency (RTL). Normal control animals entered closed arms quickly and mean RTL was shorter when compared to its own ITL. In contrast, 3-NP treated rats performed poorly and showed an increased mean RTL, compared to its own ITL. This indicates there is cognitive dysfunction in 3-NP administration. Administration of 3-NP for 14 consecutive days significantly ($p < 0.001$) decreased in the percentage of memory retention when compared to normal control rats. Treatment with alpha-pinene and Trans-anethole in 3-NP induced rats significantly ($p < 0.01$) improved the percentage of memory retention and more significantly ($p < 0.001$) improved in results were observed in the group receiving combination of alpha-pinene and Trans-anethole.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Effect of alpha-pinene and Trans-anethole on 3-NP induced changes in Forced swim test

In forced swim test, the immobility time is directly proportional to the locomotory impairment. In our study, in 3-NP induced group, there was a significant ($p < 0.001$) increase in immobility time as compared to control group. Further, alpha-pinene and Trans-anethole treatment significantly ($p < 0.01$) decreased the increase in immobility time and more significant ($p < 0.001$) results were observed in the group receiving combination of alpha-pinene and trans-anethole.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Biochemical analysis

Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in MDA levels

Lipid peroxidation estimation was performed to evaluate the oxidative stress within the animals. Administration of 3-NP for 14 consecutive days increased the MDA levels which shows that the 3-NP causes oxidative stress significantly ($p < 0.001$). Alpha-pinene and Trans-anethole significantly ($p < 0.01$) decreased the levels of Malonate dialdehyde. More significantly ($p < 0.001$) results were observed in the group receiving combination treatment of alpha-pinene and trans-anethole.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in GSH levels

Glutathione protects the brain from oxidative stress and inhibits lipid peroxidation. Alpha-pinene (AP) and trans-anethole (TA) significantly increased the levels of reduced glutathione. There was significantly ($p < 0.001$) decrease in GSH level in Huntington's disease, which was significantly ($p < 0.01$) increased by individual treatment groups and more significantly ($p < 0.001$) increased by combination group.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Effect of alpha-pinene and Trans- anethole on 3-NP induced changes in SOD levels

Administration of 3-NP for 14 consecutive days significantly ($p < 0.001$) decreased the levels of SOD which shows that the 3-NP contributes to lipid peroxidation as well as oxidative stress. Alpha-pinene and Trans-anethole significantly ($p < 0.01$) increased the levels of super oxide dismutase. More significantly ($p < 0.001$) results were observed in the group receiving combination treatment of alpha-pinene and trans-anethole compared to 3-NP alone administered group.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

Effect of alpha-pinene and trans-anethole on 3-NP induced changes in Catalase levels

Administration of 3-NP for 14 consecutive day significantly ($p < 0.001$) decreased the levels of Catalase which shows that the 3-NP contributes to oxidative stress. Alpha-pinene and trans-anethole significantly ($p < 0.01$) increased the levels of Catalase. More significantly ($p < 0.001$) results were observed in the group receiving combination treatment of alpha-pinene and trans-anethole compared to 3-NP alone administered group.

Values are expressed in Mean ± SD (n = 6). $^{***}p < 0.001$, $^{**}p < 0.01$, $^{*}p < 0.05$. Where, a indicates statistic difference from control group and b indicates statistic difference from 3-NP group. (One way ANOVA followed by Tukey's Post hoc test).

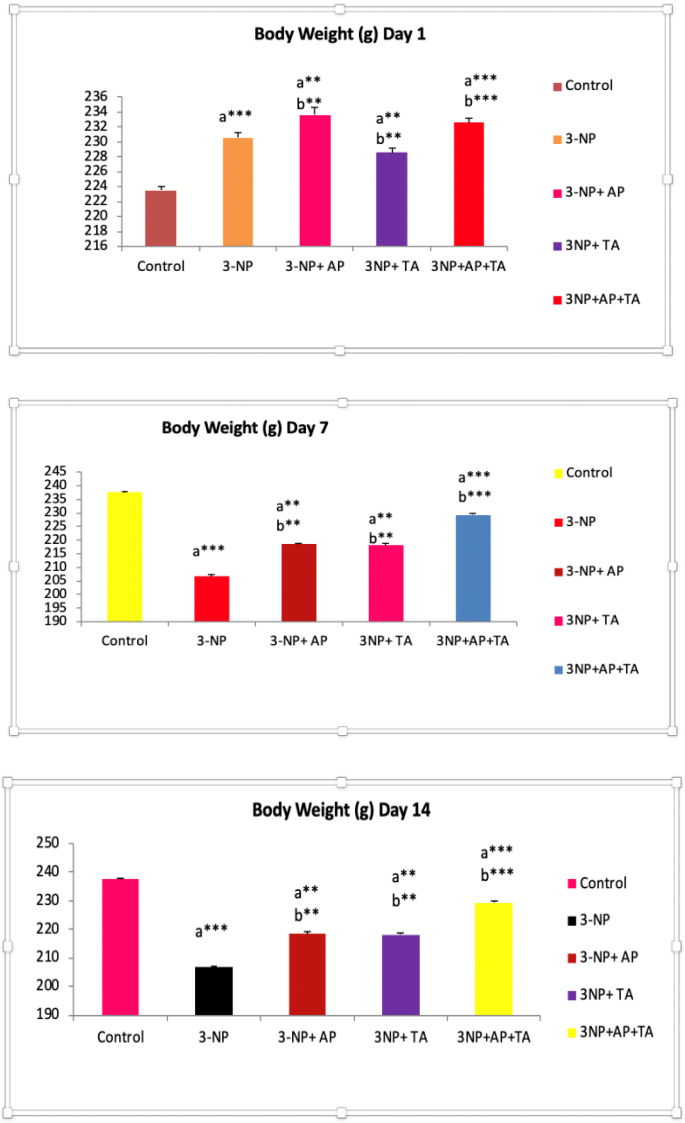

Figure 1: Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in body weight

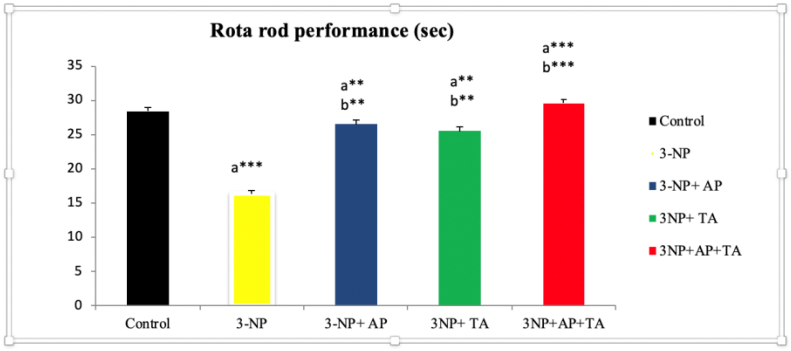

Figure 2: Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in Grip strength

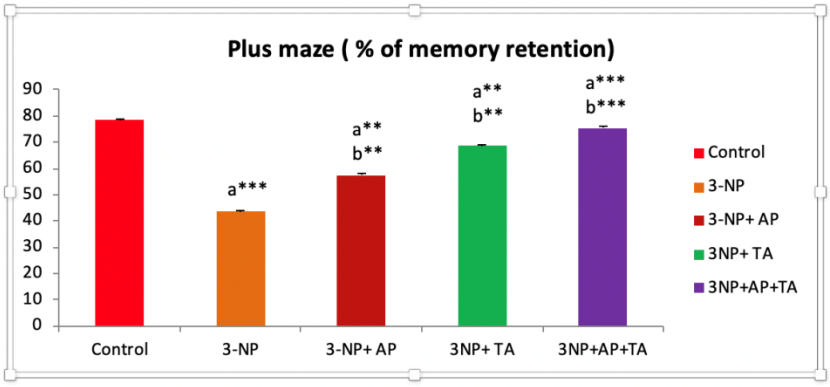

Figure 3: Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in memory retention (anxiety)

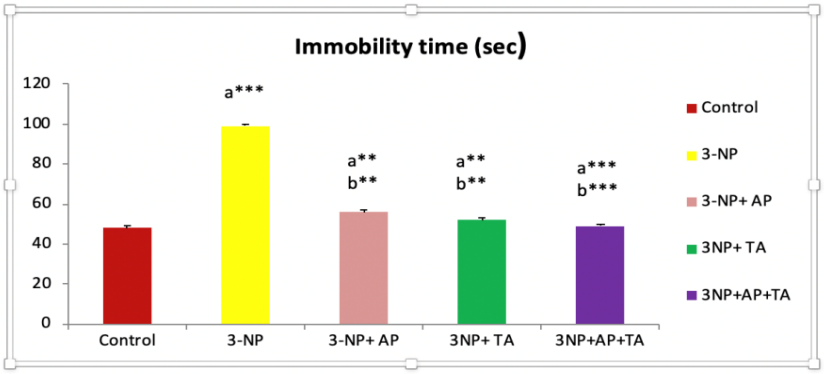

Figure 4: Effect of alpha-pinene and Trans-anethole on 3-NP induced changes in Forced swim test

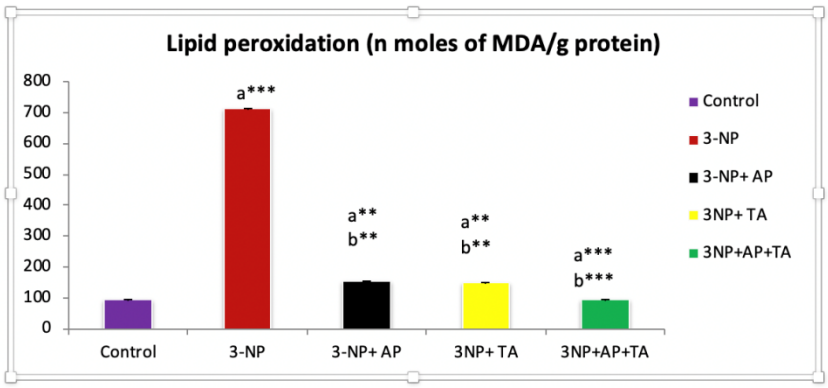

Figure 5: Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in MDA levels

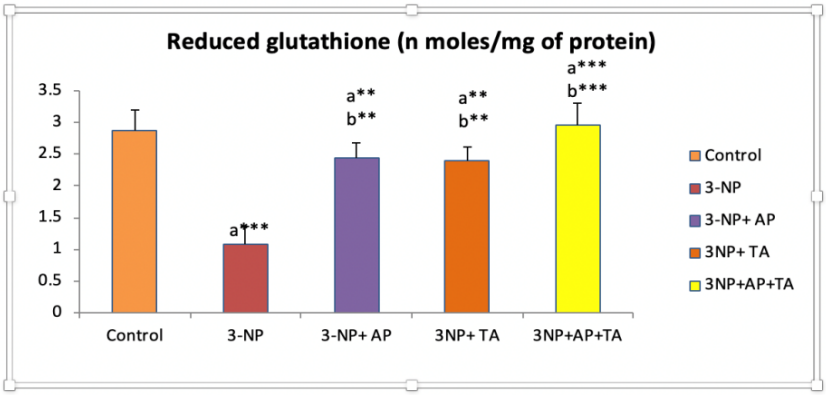

Figure 6: Effect of Alpha-pinene and Trans-anethole on 3-NP induced changes in GSH levels

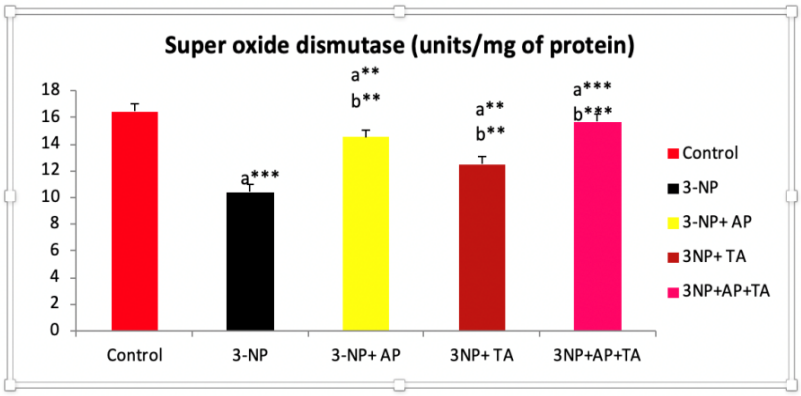

Figure 7: Effect of alpha-pinene and Trans- anethole on 3-NP induced changes in SOD level

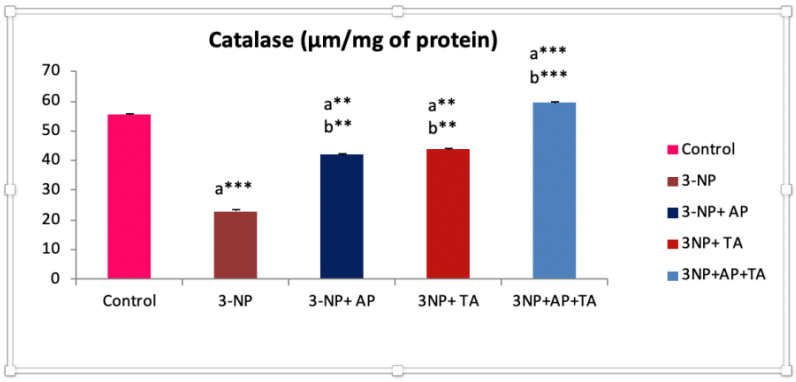

Figure 8: Effect of alpha-pinene and trans-anethole on 3-NP induced changes in Catalase levels

DISCUSSION

Huntington's disease is a disorder in which nerve cells in certain parts of the brain waste away, and degenerate. Huntington's disease causes movement, psychiatric and cognitive difficulties. In Huntington's disease increase in oxidative stress and neuronal loss alters neurotransmitters that regulate mood results in depression and anxiety. In Huntington's disease, there is a continual life change, which may be one of the sources of anxiety.

Strong evidences suggest involvement of energy impairment, excitotoxic processes and apoptosis worsen the symptoms of Huntington's disease. Striatum and hippocampus are more affected because nerve cells of the striatum are first to die as Huntington's disease progresses. Huntington's disease is not a prevalent within any particular population, races, ethnic groups, sexes. Epidemiology of Huntington's disease is less as compared to other disease, but increasing prevalence suggests that there is a new scope for further research on Huntington's disease.

Current medical therapies use pharmaceuticals interventions with lifestyle modification to prevent or control Huntington's disease. Various hypothesis including molecular genetics, oxidative stress, excitotoxicity, metabolic dysfunction and mitochondrial impairment have been proposed to explain the pathogenesis of Huntington's disease despite to that there is no treatment available fully to stop the progression of the disease.

It is well known that increased oxidative stress contribute significantly for Huntington's disease progression. Thus, pharmacological tools, as 3-NP have been employed to produce Huntington's disease in animal model. Systemic administration of 3-NP induces important increase in free radicals formation, leading to degenerative process in striatal region and Huntington's disease development.

In present study 3-NP model was used to induce Huntington's disease like symptoms in Wistar rats. 3-NP was administered i. p (10 mg/kg) for 14 days. Alpha-pinene (50 mg/kg, p. o) and Trans-anethole (50 mg/kg, p. o) and their combination (25 mg/kg, p. o, each) were taken as treatment drugs. Measurement of body weight were evaluated on day 1, day 7 and day 14 and Behavioural parameters (grip strength, elevated plus maze testing and forced swim test were analysed. Body weight was found to be significantly ($p < 0.001$) decreasing on day 7 and day 14 in Huntington's disease group, while the condition was reversed at the significant difference of ($p < 0.01$) by individual action of drugs and combination was found to be more effective at significant difference of ($p < 0.001$) in case of treatment groups. Motor co-ordination of animals was estimated by evaluating their grip strength on rota rod. Grip strength of animals in Huntington's disease group was found to be highly reduced ($p < 0.001$). It was observed to be increased ($p < 0.01$) individually and combination ($p < 0.001$) in treatment groups. Percentage of memory retention was also evaluated in Huntington's disease group 3-NP act at significant difference of ($p < 0.001$) and treatment groups individually at ($p < 0.01$) and combination at ($p < 0.001$) was found to give better results also in this study. In Forced swim test in 3-NP group was found to increased immobility at significant difference of ($p < 0.001$) and it were reduced by individually at ($p < 0.01$) and in combination it was more effective it reduced at ($p < 0.001$).

Biochemical parameters were altered due to 3-NP administration. MDA level was increased in Huntington's disease group at significantly ($p < 0.001$) which was significantly ($p < 0.01$) reduce by individual treatment groups and more significantly (p

< 0.001) reduced by combination group. Levels of anti-oxidant enzymes were decreased by 3-NP which show the oxidative stress. There was significant ($p < 0.001$) decrease in GSH level in Huntington's disease, which was significantly ($p < 0.01$) increased by individual treatment groups and more significantly ($p < 0.001$) increased by combination group. Level of SOD also found to retarding significantly ($p < 0.001$) in case of 3-NP group which was significantly ($p < 0.01$) increased by individual treatment groups and more significantly ($p < 0.001$) increased by combination group. Level of Catalase also found to retarding significantly ($p < 0.001$) in case of 3-NP group which was significantly ($p < 0.01$) increased by individual treatment groups and more significantly ($p < 0.001$) increased by combination group.

The observation of comparative effects of all the groups taken in this study shows that both Alpha- pinene and Trans-anethole are effective in reversing the Huntington's Disease like symptoms caused by 3-nitro propionic acid. The results observed also implies that the combination effect of Alpha-pinene and Trans-anethole were found to be more effectively able to give better result in the reducing the symptoms of Huntington's disease.

CONCLUSION

In the present study administration of 3-NP for 14 days, produced oxidative stress (increased levels of LPO as evident by increased MDA levels) and depleted levels of endogenous antioxidant enzyme (Catalase, reduced glutathione, SOD levels). These results supports the oxidative stress based theory of neurotoxicity caused by 3-NP. The mitochondrial dysfunction due 3-NP has lead to decreased level of succinate dehydrogenase (SDH), which is believed to be major mechanism of 3-NP toxicity.

Treatment with drugs Alpha-pinene and Trans-anethole have significantly reversed the effects of 3-NP. The treatments groups elevated the levels of GSH, Catalase and SOD and reduced the lipid peroxidation (MDA level). The weight of rats in treatments groups were also found to be increased as compared to the Huntington's disease group. However, the combination effects of drugs were found to be more effective than Alpha-pinene and Trans-anethole used in alone.

The main findings of the present study were:

i. 3-NP systemic administration in rats produces neurobehavioral and biochemical changes which were similar to that of Huntington's disease.
ii. The treatment of rats with alpha-pinene and Trans-anethole produced a neuroprotective effect and were able to reducing the symptoms of Huntington's disease progression, through this research; I am able to conclude that the combination of alpha-pine and trans-anethole can be given as treatment drug for patients of Huntington's disease.

REFERENCES

1. M. C. Chiang, Y. Chern and R.N. Huang. Gamma rescue of the mitochondrial dysfunction in Huntington's disease, Neurobiology of Disease 2012; 45(1): 322-328.
2. C.A. Ross, E.H. Aylward, E.J. Wild *et al*. Huntington disease: natural history, biomarkers and prospects for therapeutics, Nature Reviews Neurology 2014; 10(4): 204-216.
3. Bates GP, Ray D, Gusella JF, Hayden MR, Kay C, Leavitt BR, Nance M, Ross CA, Scahill RI, Wetzel R, Wildand EJ, Tabrizi SJ. Huntington disease. Nat Rev Dis Primers; 2015. p. 1-2.
4. Moily NS, Kota LN, Anjanappa RM, Venugopal S, Vaidyanathan R, Pal P, Meera Purushottam M, Jain S, Kandasamy Trinucleotide Repeats and Haplotypes at the Huntingtin Locus in an Indian Sample Overlaps with European Haplogroup A. Genetics; 2014. p. 213-234.
5. Harjes P, Wanker EE. The hunt for huntingtin function: interaction partners tell many different stories. Trends in biochemical sciences; 2003. p. 425-433.
6. Schulz JB, Henshaw DR, Macgarvey U, Beal MF. Involvement of oxidative stress in 3-nitropropionic acid neurotoxicity. Neurochemistry International; 1999. p. 167-171.
7. Salikutty Joseph and Peter K.V. Curry leaf (*Murraya koenigii*), perennial, nutritious, leafy vegetable, Economic Botany; 2008. p. 68-73.
8. Rana VS, Juyal JP, Rashmi, *et al*. Chemical constituents of the volatile oil (essential oil) of *Murraya koenigii* leaves. Int J Aromather; 2004. p. 23–25.
9. Badgujar SB, Patel VV, Bandivdekar AH. *Foeniculum vulgare* Mill: a review of its botany, phytochemistry, pharmacology, contemporary application and toxicology. Biomed Res Int; 2014. p. 842-674.
10. Swaroop, Banerjee S, Handral M. Neuroprotective Evaluation of Leaf Extract of *Dalbergia sissoo* in 3-Nitropropionic Acid Induced Neurotoxicity in Rats. International Journal of Pharmaceutical Sciences and Drug Research; 2014. p. 41-47.
11. Porsolt R.D. Behavioural despair in rats. A new model sensitive to antidepressant treatments; 1978. p. 379-391.
12. Kumar P, Kalonia H, Kumar A. Sesamol attenuate 3-nitropropionic acid-induced Huntington-like behavioural, biochemical and cellular alterations in rats. Journal of Asian Natural Products Research; 2009. p. 439-450.
13. Ohkawa H, Ohishi N, Yagi K. Assay of lipid peroxides in animal tissue by thiobarbituric acid reaction. Anal Biochem; 1979. p. 351-359.
14. Moron MS, Depierre JW, Mannervik. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta; 1979. p. 67–78.
15. Kakkar P, Das B, Viswanathan PN. A modified spectrophotometric assay of superoxide dismutase; 1994. p. 130-132.
16. Aebi H *et al*. Catalase, method in enzyme analysis vol - II, (Ed. H.U, Bergmer); 1974. p. 673-680.

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